Remarks

Claims 33, 37-41, 45, 49, and 50 were pending in the subject application. By this Amendment, claims 33 and 50 have been amended. The undersigned avers that no new matter is introduced by this amendment. Entry and consideration of the amendments presented herein is respectfully requested. It should be understood that the amendments presented herein have been made solely to expedite prosecution of the subject application to completion and should not be construed as an indication of the applicants' agreement with or acquiescence in the Examiner's position. Claims 37-41, 45, and 49 remain pending but withdrawn from consideration. Accordingly, claims 33 and 50 are currently before the Examiner for consideration. Favorable consideration of the pending claims is respectfully requested.

The applicants and the applicants' representative wish to thank Examiner Hill and Supervisory Examiner Housel for the courtesy of the telephonic interview conducted with the undersigned on January 26, 2006. Claims 33 and 50, as previously pending and as currently amended, and the rejections under 35 U.S.C. §103(a) were discussed. The undersigned asserted that the Patent Office had not established a *prima facie* case of obviousness. Supervisory Examiner Housel invited the applicants to submit evidence of unexpected results. The remarks and amendments set forth herein are consistent with the substance of the interview and are believed to address the outstanding issues as discussed during the interview.

Submitted herewith is a supplemental Information Disclosure Statement (IDS), accompanied by the form PTO/SB/08 and copies of the references listed therein. The applicants respectfully request that the references listed on the form PTO/SB/08 be considered and made of record in the subject application.

By this Amendment, the applicants have amended claims 33 and 50 to recite that the plasmid DNA are expressed *in vivo*, thereby producing each of said RSV antigens. Support for these amendments can be found, for example, at page 4, lines 3-4, of the specification, which indicates that the vaccine induces antibodies to nine antigens.

Claim 33 has been rejected under 35 U.S.C. §103(a) as being obvious over Connors *et al.* (*J. Virol*, 1991, 65(3):1634-1637) in view of Li *et al.* (*J. Exp. Med.*, 1998, 188(4):681-688) and Li *et al.*

(*Virology*, 2000, 269:54-65), and further in view of Leong (*J. Controlled Release*, 1998, 53:183-193). In addition, claim 50 has been rejected under 35 U.S.C. §103(a) as being obvious over Connors *et al.* in view of Li *et al.* (1998) and Li *et al.* (2000) and Leong, and further in view of Illum (WO 90/09780) or Rolland *et al.* (U.S. 6,184,037) or Wyatt (*Vaccine*, 1999, 18:392). The applicants respectfully submit that the immunogenic composition as currently amended is not obvious over the cited references.

As indicated above, by this Amendment, the applicants have amended claims 33 and 50 to recite that the plasmid DNA are expressed *in vivo*, thereby producing each of said RSV antigens. At page 4, the Office Action indicates that the specification does not show expression of all nine RSV antigens. The applicants submitted the Kumar *et al.* publication (*Human Gene Therapy*, August 2002, 13:1415-1425) with the Information Disclosure Statement on May 16, 2005, which was considered by the Examiner. As discussed during the telephonic interview, Figure 1A of the Kumar *et al.* publication shows expression of RSV cDNAs encoding all nine antigens after intranasal administration to mice, as determined by RT-PCR analysis. The F antigen is faint, but present (upper third portion of the lane). Furthermore, the immunoblot analysis in Figure 1B of the Kumar *et al.* publication confirms that the mRNA of several of the antigens, including the F antigen (F1 and F2 subunits), are translated and elicit an antibody response (see Figures 1A-1B and first paragraph of Results section at page 1419).

Any rejection of a claim for obviousness must include a finding that one of ordinary skill in the art at the time the invention was made would have reasonably expected the claimed invention to work. *Hodosh v. Block Drug Co.*, 786 F2d 1136 (Fed Cir 1986); *In re Merck & Co., Inc.*, 800 F.2d 1091; 231 USPQ 375 (Fed. Cir. 1986). The applicants respectfully submit that the cited references would not impart any reasonable expectation of success to one of ordinary skill in the art. The applicants submitted several scientific publications with their previous Amendment of May 16, 2005 showing that the immune response to a composition employing a combination or "cocktail" of antigens cannot be predictably determined from responses obtained from the same antigens individually.

The cited references, alone or in combination, do not establish a reasonable expectation of success in combining the references to arrive at the invention. The Conners *et al.* publication, which

is relied upon as the primary reference in each of the foregoing rejections under 35 U.S.C. §103(a), describes experiments evaluating whether nine vaccinia virus-RSV recombinants individually encoding nine RSV proteins are able to induce resistance to RSV challenge. The Office Action cites the Conners *et al.* publication for teaching that each of the tested RSV proteins is capable of inducing an immune response against RSV. However, the cited reference must be taken into consideration as a whole. For example, at page 1635, second column, Conners *et al.* indicate that previous studies identified F and G glycoproteins as the major mediators of resistance to RSV infection with RSV, with the N protein providing partial protection. Further, Conners *et al.* also state that "importantly, the other RSV proteins (SH, M, P, 1B, and 1C) failed to induce resistance under the experimental conditions used." Finally, Conners *et al.* conclude that "the major antigens to be included in an RSV vaccine are the F and G glycoproteins, which efficiently stimulate neutralizing antibodies", and "RSV antigens need only contain the F and G glycoproteins, because the immunity conferred by the other proteins is less effective and appears to wane rapidly with time" (see 1636, second column, and abstract of Conners *et al.*, emphasis added). Thus, the applicants respectfully submit that the Conners *et al.* publication actually teaches away from an immunogenic composition utilizing plasmid DNA encoding seven RSV antigens in addition to F and G. A prior art reference must be considered in its entirety, *i.e.*, as a whole, including portions that would lead away from the claimed invention. *W.L. Gore & Associates, Inc. v. Garlock, Inc.*, 721 F.2d 1540, 220 USPQ 303 (Fed. Cir. 1983), *cert. denied*, 469 U.S. 851 (1984).

The Examiner has not established why one skilled in the art would include plasmids encoding antigens other than F and G, despite the added expense. This is particularly relevant given the recognized unpredictability in host immune response to RSV vaccines. As described in more detail in the Declaration under 37 C.F.R. §1.132 that accompanies this Amendment, and page 54, second column, of the Li *et al.* publication (*Virology*, 2000, 269:54-65), there is a risk of exacerbating the disease. In the early 1960s, a trial of formalin-inactivated alum-precipitated RSV (FI-RSV) was conducted. However, rather than protecting vaccinees against infection, children immunized with the FI-RSV preparation experienced more severe disease following subsequent natural exposure to the virus, resulting in the hospitalization of 80% of FI-RSV-immunized infants and two deaths, compared to 5% hospitalization and no deaths in children immunized with a similar preparation of

parainfluenza virus (Kapikian *et al.*, *Am. J. Epidemiol.*, 1969, 89:405-421; Kim *et al.*, *Am. J. Epidemiol.*, 1969, 89:422-434).

The Office Action relies on the Li *et al.* publications for teaching that the use of plasmid DNA vaccines encoding viral antigens leads to an improved immune response compared to those seen with other vaccines, and a response comparable to that observed with natural RSV infection can be achieved with plasmid vaccines encoding RSV F and G antigens, individually. The Leong *et al.* publication describes coacervating DNA with chitosan to form DNA-chitosan nanospheres to improve DNA delivery. The Illum reference is relied upon by the Office Action for teaching that chitosan particles comprising RSV vaccines may be nasally administered, and the Illum and Rolland references are relied upon in the Office Action for teaching that chitosan microparticles encapsulating DNA may be administered as an inhalant.

It is only the subject application that provides the teaching that coacervates of chitosan and plasmid DNA encoding nine RSV antigens can be synthesized and delivered to a host, wherein the antigen-encoding DNA is expressed at sufficient levels within the host's cells to achieve the desired immune response against RSV. Synthesis of nanospheres that successfully express the plasmid DNA encoding each of the nine recited antigens is dependent on several interrelated factors including, for example, plasmid concentration, chitosan concentration, the ratio of plasmid to chitosan, the molecular weight of chitosan, temperature, mode of mixing, pH, and the size and surface charge of the particles. The cited references do not establish that nanospheres comprising coacervated chitosan and plasmid DNA encoding nine RSV antigens can be used to deliver and express each antigen resulting in an effective immune response to RSV.

The ultimate determination of patentability must be based on the entire record, by a preponderance of the evidence, with due consideration to the persuasiveness of any arguments and any secondary evidence. *In re Oetiker*, 977 F.2d 1443, 24 USPQ2d 1443 (Fed. Cir. 1992). The legal standard of "a preponderance of evidence" requires the evidence to be more convincing than the evidence which is offered in opposition to it. With regard to rejections under 35 U.S.C. §103, the Examiner must provide evidence which, as a whole, shows that the legal determination sought to be proved (*i.e.*, the teachings of the references establish a *prima facie* case of obviousness) is more probable than not. The applicants respectfully submit that, based upon a preponderance of the

evidence, the cited references would not impart any reasonable expectation of success to one of ordinary skill in the art.

Submitted herewith in rebuttal to the obviousness rejections is a Declaration under 37 C.F.R. §1.132 by Dr. Shyam Mohapatra for the Examiner's consideration. Acute RSV infection is associated with episodes of bronchiolitis (inflammation of the bronchioles (small airways)), which causes wheezing and pneumonia among infants and young children. As indicated at page 14, line 16, of the subject specification, and as elaborated upon by Dr. Mohapatra in his Declaration, the potential enhancement of inflammation is a major concern with RSV vaccines. The composition of the invention significantly attenuates pulmonary inflammation induced by RSV infection (see page 3, lines 15-18, page 7, lines 11-14, and page 15, lines 1-10, of the subject specification).

As is made clear from Dr. Mohapatra's Declaration, there is inherent unpredictability in a host's immune response to RSV vaccination. Treatments for severe lower respiratory tract infection caused by RSV remain elusive. Despite decades of intense research, a safe and effective RSV vaccine that can be given to infants has eluded investigators. As indicated above, perhaps the single most important concern is that vaccination may actually exacerbate naturally occurring RSV infection, a phenomenon witnessed when the formalin-inactivated RSV vaccine was administered to infants and children during the 1960s (Chanock *et al.*, 1992; Kapikian *et al.*, *Am. J. Epidemiol.*, 1969, 89:405-421; Kim *et al.*, *Am. J. Epidemiol.*, 1969, 89:422-434; Chin *et al.*, *Am. J. Epidemiol.*, 1969, 89:449-463; Openshaw *et al.*, *Vaccine*, 2002, 20:S27-S31). Another obstacle to the development of a vaccine is that RSV infection itself, even in its most severe form, elicits incomplete immunoprotection. Infection can recur.

While common sense dictates that treatment of serious viral bronchiolitis should start with agents that interfere with virus replication, as explained by Dr. Mohapatra, "the pathogenesis of bronchiolitis depends on two co-existing events, which may not be easy to separate from one another" (Mohapatra Declaration, section 4 at page 3). First, the virus attaches to and invades the respiratory epithelial cell, which serves as the host for viral replication. The virus then spreads to adjacent areas by lysis or by fusing with neighboring cells. Next, the respiratory epithelial cells respond by releasing a repertoire of pro-inflammatory mediators that serve to recruit inflammatory cells important in controlling the viral infection (see, for example, Piedra, P.A., *Pediatr. Infect. Dis.*

J., 2003, 22:S94-S99). As indicated by Dr. Mohapatra, "this inflammatory response can be robust and difficult to control once initiated" (Mohapatra Declaration, section 4 at page 3). The inflammation associated with RSV infection has gained recent attention as a new target for intervention (see, for example, page S95, second column, second full paragraph of Piedra, 2003; and page 541, second column, first full paragraph, pages 544-545, and page 546, first two paragraphs of Openshaw and Tregoning, *Clinical Microbiology*, 2005, 18:541-555). If virus replication can be controlled, through antiviral medications or other agents, while at the same time the virus-induced inflammatory cascade can be fine-tuned, clinical improvement is expected. "Thus, a successful RSV intervention should inhibit virus replication and protect against or minimize the inflammation associated with RSV infection" (Mohapatra Declaration, section 4 at page 3).

The composition of the subject invention utilizes chitosan-DNA nanospheres containing a cocktail of plasmid DNAs (pDNAs) encoding nine immunogenic RSV antigens. The Kumar *et al.* (2002) publication describes an *in vivo* study evaluating the chitosan-DNA nanospheres of the invention as an effective and safe prophylaxis against RSV. The nanospheres were administered intranasally into the mouse lung. A single administration of nanospheres (25 μg of total DNA per mouse) induced expression of the mRNA and proteins for all nine RSV antigens in the lung and resulted in a significant reduction of viral titers and viral antigen load after acute RSV infection of these mice. The therapy also induced the production of anti-RSV antibody with neutralizing properties, enhanced interferon-gamma production in spleen and lung, and generated cytotoxic T lymphocyte responses against RSV. Importantly, this gene expression therapy also reduced RSV-induced lung inflammation. Lung inflammation was examined in different groups of mice. As shown at page 1420 of Kumar *et al.*, mice treated with chitosan alone (Figure 3A), chitosan plus the empty expression vector, pVAX (Figure 3C), naked DNA (Figure 3B), or PBS on acute RSV infection exhibit disruption of the epithelium and cellular infiltration. Representative pathological features reveal that groups of mice receiving the nanospheres of the invention exhibit less epithelial damage and reduced mononuclear cell and polymorphonuclear cell infiltrates in the interstitial and peribronchovascular regions (Figure 3D), as compared with controls (Figures 3A-3C). The arrows indicate epithelial damage and cellular infiltration. A semiquantitative analysis using a scoring system for inflammation in the lung is shown in Table 2 at page 1421 of Kumar *et al.* The scores for

epithelial damage, interstitial-alveolar infiltrate, and peribronchovascular infiltrate are significantly lower ($p < 0.001$ to $p < 0.05$) for mice that received the nanospheres, compared with controls. As indicated by Dr. Mohapatra, "these results are strong evidence that the nanospheres provide protection from RSV infection-induced pulmonary inflammation" (Mohapatra Declaration, section 5 at page 4). The immunologic mechanisms for the effectiveness of the nanospheres' prophylaxis include the induction of high levels of both serum IgG and mucosal IgA antibodies, the generation of an effective CTL response, and elevated lung-specific production of IFN-gamma with antiviral action. Importantly, the nanospheres also decrease pulmonary inflammation and do not alter airway hyperresponsiveness, making them a safe vaccine against RSV.

"The robust anti-inflammatory activity exhibited by the nanospheres of the invention is unexpected based on the references cited in the Office Action, and is particularly advantageous for RSV intervention for the reasons discussed above" (Mohapatra Declaration, section 6 at page 5). The Li *et al.* publication (*J. Exp. Med.*, 1998, 188(4):681-688) evaluates pulmonary inflammation of mice immunized with plasmids encoding the RSV F protein (pXL2) intramuscularly (i.m.) and intradermally (i.d.) after RSV challenge. Results were compared with that of mice administered with RSV (intranasally), formalin-inactivated RSV (FI-RSV) (i.m.), and empty vector (pXL0) (i.m.). As shown in Table 3 at page 686, mice immunized with the pXL0 control had a mild pulmonary inflammatory reaction (135 +/- 0.60) compared with pXL2, RSV and FI-RSV. In fact, mice immunized with pXL2 had a significantly more intense inflammation of the bronchioles than the control (pXL0) and RSV itself.

The Office Action cites the Leong *et al.* publication for showing that coacervation with chitosan can improve delivery of plasmid DNA into cells. However, as stated by Dr. Mohapatra "most of the informative data in the Leong *et al.* publication pertains to DNA complexed with gelatin, instead of chitosan" (Mohapatra Declaration, section 7 at page 5). The data presented in the Leong *et al.* publication show that gelatin and chitosan perform differently as gene delivery vehicles. For example, Figure 5 at page 188 of Leong *et al.* demonstrates that luciferase transfection of 293 cells with DNA-gelatin nanospheres resulted in a transfection efficiency that was clearly dose-responsive. This transfection efficiency was not evident in the DNA-chitosan nanospheres. Both nanospheres were less effective than lipofectamine. Moreover, the results obtained with HEK293

cells in the Leong *et al.* publication cannot necessarily be extrapolated to the target cells of an RSV vaccine. As explained by Dr. Mohapatra,

> ...the HEK293 cells used in these studies represent an easily transfectable cell line and studies using this cell line are not reflective of other lung epithelial cell lines, such as lung alveolar type-2 A549 epithelial cells and normal bronchial epithelial cells, which are relevant to RSV. Differences in transfectability among cell lines, which was observed in the Leong *et al.* publication, are common place in *in vitro* transfection studies. (Mohapatra Declaration, section 7 at page 5)

Figure 7 in the Leong *et al.* publication shows intracellular distribution of DNA-gelatin nanospheres, not DNA-chitosan nanospheres. Furthermore, the penultimate paragraph at page 192 of the Leong *et al.* publication indicates that "these data suggest that the DNA-chitosan nanospheres might be entering the cells through a mechanism different from that used by the DNA-gelatin nanospheres" (Mohapatra Declaration, section 7 at page 6). The last paragraph at page 192 of the Leong *et al.* publication references a citation reporting successful delivery of the CFTR gene to rabbit lung airways but this citation [24] pertains to gelatin nanospheres. Likewise, the last sentence references citation [25] as suggesting that the DNA-nanospheres "might be attractive vehicles for DNA vaccine applications". This citation also pertains to gelatin nanospheres.

The effectiveness of the nanospheres of the invention, particularly with respect to their ability to decrease RSV-induced pulmonary inflammation, is unexpected in view of the cited references. "A greater than expected result is an evidentiary factor pertinent to the legal conclusion of obviousness ... of the claims at issue." *In re Corkill*, 711 F.2d 1496, 226 USPQ 1005 (Fed. Cir. 1985). Evidence of unobvious or unexpected advantageous properties, such as superiority in a property the claimed compound shares with the prior art, can rebut *prima facie* obviousness. "Evidence that a compound is unexpectedly superior in one of a spectrum of common properties . . . can be enough to rebut a prima facie case of obviousness." No set number of examples of superiority is required. *In re Chupp*, 816 F.2d 643, 646, 2 USPQ2d 1437, 1439 (Fed. Cir. 1987).

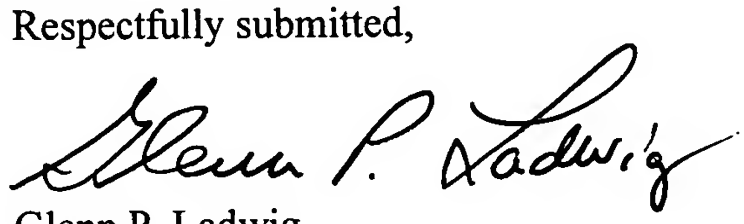
The applicants note that, in section 2 at page 2 of Dr. Mohapatra's Declaration, the Kneyber *et al.* publication is cited incorrectly. The correct citation is Kneyber *et al.*, *Eur. J. Pediatr.*, 2000, 159:399-411.

In view of the foregoing remarks and amendments to the claims, the applicants believe that the currently pending claims are in condition for allowance, and such action is respectfully requested.

The Commissioner is hereby authorized to charge any fees under 37 C.F.R. §§ 1.16 or 1.17 as required by this paper to Deposit Account 19-0065.

The applicants invite the Examiner to call the undersigned if clarification is needed on any of this response, or if the Examiner believes a telephonic interview would expedite the prosecution of the subject application to completion.

Respectfully submitted,



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Supplemental Information Disclosure Statement
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